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Research report

The potential anti-addictive agent, 18-methoxycoronaridine, blocks the sensitized locomotor and dopamine responses produced by repeated morphine treatment

Karen K. Szumlinski*, Isabelle M. Maisonneuve, Stanley D. Glick

Department of Pharmacology and Neuroscience (MC-136), Albany Medical College, 47 New Scotland Avenue, Albany, NY, 12208, USA

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Abstract

18-Methoxycoronaridine (18-MC), a novel synthetic iboga congener, attenuates the reinforcing efficacy of morphine, disrupts some signs of morphine withdrawal in physically dependent rats and attenuates the dopamine response in the nucleus accumbens to acute morphine. The present study further investigated the interactions between 18-MC and morphine by examining the effects of 18-MC (40 mg/kg, i.p., 19 h earlier) on the expression of dopamine sensitization in the nucleus accumbens in response to morphine (20 mg/kg, i.p.) and on the dose–effect curves for morphine-induced locomotion (0–30 mg/kg, i.p.) in rats treated either acutely or repeatedly (five, once daily, injections of 20 mg/kg, i.p.) with morphine. Compared to vehicle pretreated controls, 18-MC increased the potency of morphine, shifting the dose–response curve to the left, in acute morphine treated rats; however, 18-MC did not alter the potency of morphine in rats treated repeatedly with morphine. Repeated morphine administration induced locomotor sensitization in approximately 50% of the rats tested; in vehicle pretreated rats, the morphine dose–response curve was shifted to the left in sensitized as compared to non-sensitized rats. In 18-MC pretreated rats, sensitized and non-sensitized rats responded similarly to morphine, revealing a blockade of sensitization by 18-MC. Consistent with this behavioural finding, 18-MC pretreatment completely abolished the sensitized dopamine response in the nucleus accumbens expressed by rats repeatedly treated with morphine. It is suggested that the potential anti-addictive efficacy of 18-MC might be related to an ability to restore normal functioning to a hypersensitive mesolimbic dopamine system produced by previous repeated morphine administration. © 2000 Elsevier Science B.V. All rights reserved.

Themes: Disorders of the nervous system and aging

Topics: Drugs of abuse: opioids and others

Keywords: 18-Methoxycoronaridine (18-MC); Morphine; Sensitization; Locomotor activity; Dopamine; Drug addiction

1. Introduction

18-Methoxycoronaridine (18-MC), a synthetic iboga alkaloid congener, derived from the putative anti-addictive drug, ibogaine, was developed with the goal of making available a safer ibogaine-like agent. Similar to ibogaine, and other iboga alkaloid derivatives [15,16,46,47], 18-MC effectively decreases the self-administration of several drugs of abuse in laboratory animals [15,16,18–20,49]. Also similar to ibogaine [20], 18-MC attenuates acute

signs of morphine withdrawal in physically dependent rats [49]. However, unlike ibogaine [16,38–40], 18-MC is non-tremorigenic, does not depress operant responding for water and, when administered at doses several times higher than doses which effectively decrease drug self-administration (i.e., 300 vs. 40 mg/kg), does not induce cerebellar neurotoxicity or bradycardia, for reviews see Refs. [18,19].

In terms of a mechanism to account for 18-MC's anti-addictive effects, 18-MC, when administered alone, produces similar decreases in extracellular levels of dopamine in the nucleus accumbens as those produced by other iboga agents that decrease drug self-administration in rats [15–18,46,47]. This common action may underlie the effects of these potential anti-addictive compounds on the

*Corresponding author. Tel.: +1-518-262-5303; fax: +1-518-262-5799.

E-mail address: kszumlinski@ccgateway.amc.edu (K.K. Szumlinski)

reinforcing or incentive motivational properties of abused drugs (e.g. [5,14,31,32,59]). Consistent with this possibility, recent work in our laboratory demonstrated that 18-MC produces a greater attenuation of dopamine in the shell of the nucleus accumbens, compared to the dorsolateral striatum, in response to an acute injection of morphine [34].

Currently, nothing is known about the interactions between 18-MC and morphine with respect to the behaviour of animals repeatedly treated with morphine. However, previous studies using ibogaine demonstrated that ibogaine produces a greater attenuation of morphine-induced locomotion in rats treated repeatedly, versus acutely, with morphine [43]. Such findings indicate that repeated morphine administration increases an animal's sensitivity to the effects of ibogaine on morphine-induced locomotion. The repeated, intermittent, administration of morphine can induce a progressive decrease in the latency to onset of morphine's locomotor-activating effects [1,4,13,24,25,58], an effect putatively mediated by an enhancement in mesotelencephalic dopamine (for review see [29]). Termed, sensitization (for review, [50]), this phenomenon has been theorized by some authors to lie at the very core of both drug craving and relapse in drug addiction, e.g., [28,51]. Thus, the possibility exists that the ability of ibogaine-related compounds, such as 18-MC, to attenuate morphine self-administration and withdrawal might involve alterations in the expression of morphine-induced sensitization. The present study investigated the influence of 18-MC on morphine sensitization by examining the effects of 18-MC on morphine-induced locomotion in rats treated either acutely or repeatedly with morphine and the effects of 18-MC on dopamine transmission in the nucleus accumbens in repeated morphine treated rats.

2. Methods

2.1. Animals

Subjects were naïve female Sprague–Dawley rats (Taconic, Germantown, NY, USA), weighing 175–225 g (locomotor experiments) or 250–275 g (microdialysis experiment). All animals were housed in polyurethane cages in a colony room controlled for temperature and humidity, maintained on a normal light/dark cycle (lights on/off at 0700 h/1900 h). Food and water were available ad libitum.

2.2. Drugs

18-Methoxycoronaridine hydrochloride, obtained from Albany Molecular Research (Albany, NY, USA), was dissolved in phosphate buffer (VEH) and injected i.p. at a concentration of 20 mg/ml (volume=2 ml/kg). Morphine sulfate, purchased from Research Biochemicals (Natick,

MA, USA), was dissolved in saline and injected i.p. at the particular concentrations (0.075–30 mg/ml) required for each experiment (volume=1 ml/kg).

2.3. Locomotor activity procedures

The effects of pretreatment with 18-MC on morphine-induced locomotion were assessed in two separate experiments. For the study of the effects of 18-MC on acute morphine-induced locomotion, rats were randomly assigned to receive 18-MC (40 mg/kg; i.p.) or VEH and then, 19 h later, were injected with one of seven test doses of morphine (0, 0.075, 1.25, 5, 10, 20 or 30 mg/kg). For the study of the effect of 18-MC on the expression of locomotor sensitization following repeated morphine treatment, rats were treated with five, once daily, injections of morphine (20 mg/kg; i.p.). 18-MC or VEH was administered 48 h later and this injection was followed, 19 h later, by a morphine dose–response test (0, 5, 10, 30 mg/kg). The time-course of morphine-induced locomotion is biphasic, with locomotor stimulation preceded by locomotor depression, e.g., [1]. Rats were identified as sensitized or non-sensitized based on whether, or not, the latency to onset of morphine-induced locomotor activation advanced in time from injection 1 to injection 5 of repeated morphine treatment. This was done by an experimenter blind to the test group assignment of the animals.

For both experiments, rats were immediately placed in opaque cylindrical photocell activity cages (diameter 60 cm, three crossing beams) following morphine injection where their locomotor activity was monitored for 3 h. Interruptions of two light beams in succession were recorded as one activity count by a PC computer using the software MED-PC (Med Associate, St. Albans, VT, USA). 18-MC/VEH injections were administered in the colony room and rats were returned immediately to their home cages following injection.

2.4. In vivo microdialysis procedure

2.4.1. Surgery

Under pentobarbital anesthesia (50 mg/kg, i.p.), rats were stereotactically implanted with two microdialysis guide cannulae (CMA: 8309010; Acton, MA, USA) aimed bilaterally, at an angle of 14°, over the nucleus accumbens. The coordinates were chosen such that, when inserted, the tips of the dialysis probes were located in the medial portion of the shell area of the accumbens (AP, +1.6 mm and L, $\pm$ 3.0 mm, V, –8.8 mm from the surface of the skull) [41]. Animals were monitored for proper recovery but otherwise left undisturbed for 4 days after surgery.

2.4.2. Repeated morphine treatment

The afternoon prior to Injection 1 of repeated morphine treatment (the first microdialysis session), the rats were

briefly anesthetized with Brevital (45 mg/kg, i.p.) and a dialysis probe (CMA 8309502) was inserted through the guide cannula on one side of the head. Artificial CSF (146 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl_2 and 1.0 mM MgCl_2) was delivered by a Harvard syringe pump at a flow-rate of 1 $\mu\text{l}/\text{min}$. Collection of perfusates began the next day (11:00 h). Twenty-minute fractions were collected in vials containing 2.0 μl of 1.1 M perchloric acid solution (containing 50 mg/l EDTA and 50 mg/l sodium metabisulfite). After 2 h of baseline collections, the rats received their first injection of morphine (20 mg/kg, i.p.) (13:00 h). The collection of dialysate samples was then continued for 3 h. Following completion of the first microdialysis session, rats were briefly anesthetized as described above, the probes were removed, and the animals were returned to the colony room in home cages. On each of the next four morphine injection days, rats were transported to the experimental room in their home cages (13:00 h), injected with morphine (20 mg/kg, i.p.), and then returned immediately to their home cages. Home cages were placed on shelves either above, or below, the microdialysis chambers for 3 h, at the end of which time, rats were returned to the colony room. This repeated morphine treatment procedure was conducted to allow for the formation of morphine-context associations which promote the expression of dopamine sensitization on test day [56].

2.4.3. Test injection

Following 48 h of withdrawal, a microdialysis probe was inserted into the opposite guide cannula as that for Injection 1 (counterbalancing sides across pretreatment groups); otherwise identical procedures for probe insertion and perfusion were followed as described above. Rats received either 18-MC (40 mg/kg; i.p.) or VEH pretreatment ~1 h following probe insertion (~19:00 h) such that pretreatment occurred exactly 19 h prior to the test injection of morphine (20 mg/kg). Baseline sampling, morphine injection and test sampling procedures were identical to those described above. Upon completion of the test session, rats were sacrificed by an overdose of pentobarbital, and their brains were removed, frozen, and sliced (40 μm coronal sections) in a cryostat. The tracks left by the probes were identified and their exact positions determined by reference to the atlas of Paxinos and Watson [41]. Only the dialysates of animals whose tracks were within 0.5 mm of the correct placement were analyzed.

2.5. Catecholamine assay

Dialysate samples were assayed for dopamine, DOPAC, and HVA by HPLC with electrochemical detection. The HPLC system consisted of an ESA 540 autosampler, an ESA 580 solvent delivery system, an ESA small bore column (MD-150/RP-C18; diameter=3.0 μm) and an ESA Coulochem II electrochemical detector with a work-

ing electrode set at a potential of 250 mV with respect to a reference electrode. The mobile phase was purchased from ESA (MD-TM) and consisted of 0.075 μM sodium dihydrogenphosphate, monohydrate, 0.0017 μM 1-octanesulfonic acid, 25 μM EDTA in 10% HPLC grade acetonitrile, and was adjusted to pH 3 with phosphoric acid. The flow-rate was set at 0.53 ml/min. Sample chromatograms are provided in Fig. 1. Chromatograms were integrated using Hewlett–Packard CHEMSTATION software.

2.6. Statistical analysis

For the acute morphine locomotion experiment, data were examined for main effects by analysis of variance (ANOVA) for the between subjects factors of Pretreatment (18-MC vs. VEH) and Dose (0, 0.05, 1.25, 5, 10, 20 and 30 mg/kg) and the within subjects factor of Time (0–180 min). For the repeated morphine locomotion experiment, data were examined by ANOVA for the between subjects factors of Group (sensitized vs. non-sensitized), Pretreatment (18-MC vs. VEH), Dose (0, 5, 10 and 30 mg/kg) and the within subjects factors of Injection Number (1–5) and Time (0–180 min). For the microdialysis experiment, baseline data, expressed as pm/10 μl , were examined by ANOVA for the between subjects factor of Pretreatment (18-MC vs. VEH) and the within subjects factors of Session (injection 1 vs. test) and Time (0–120 min). The morphine data were expressed as percent of baseline and examined by ANOVA for the between subjects factor of Group (Injection, VEH or 18-MC) and the within subjects factor of Time (0–300 min). If there were significant effects, the factors were decomposed and Duncan's multiple-range post-hoc tests were performed (STATISTICA).

3. Results

3.1. Acute morphine locomotor study

Morphine dose-dependently induced locomotor responding in both VEH and 18-MC pretreated rats during the 3-h test session [Dose effect: $F(6,155)=23.57$, $P<0.0001$; Dose $\times$ Time interaction: $F(102, 2635)=29.50$, $P<0.0001$]. As can be observed in Figs. 2 and 3, pretreatment with 40 mg/kg 18-MC, 19 h earlier, altered the acute dose–response relationship for morphine-induced locomotion [Pretreatment $\times$ Dose interaction: $F(6,155)=2.61$, $P<0.03$; Pretreatment $\times$ Dose $\times$ Time interaction: $F(102,2653)=1.79$, $P<0.0001$]. 18-MC shifted the dose–response function to the left of VEH-pretreated controls; 18-MC pretreated rats displayed maximal locomotor responding to both 5 and 10 mg/kg morphine [Dose Effect: $F(6,80)=16.20$, $P<0.0001$, post-hoc tests], whereas maximal locomotor responding was apparent only at the 10 mg/kg dose in controls [dose effect: $F(6,75)=9.43$, $P<0.0001$, post-hoc tests] (Fig. 2).

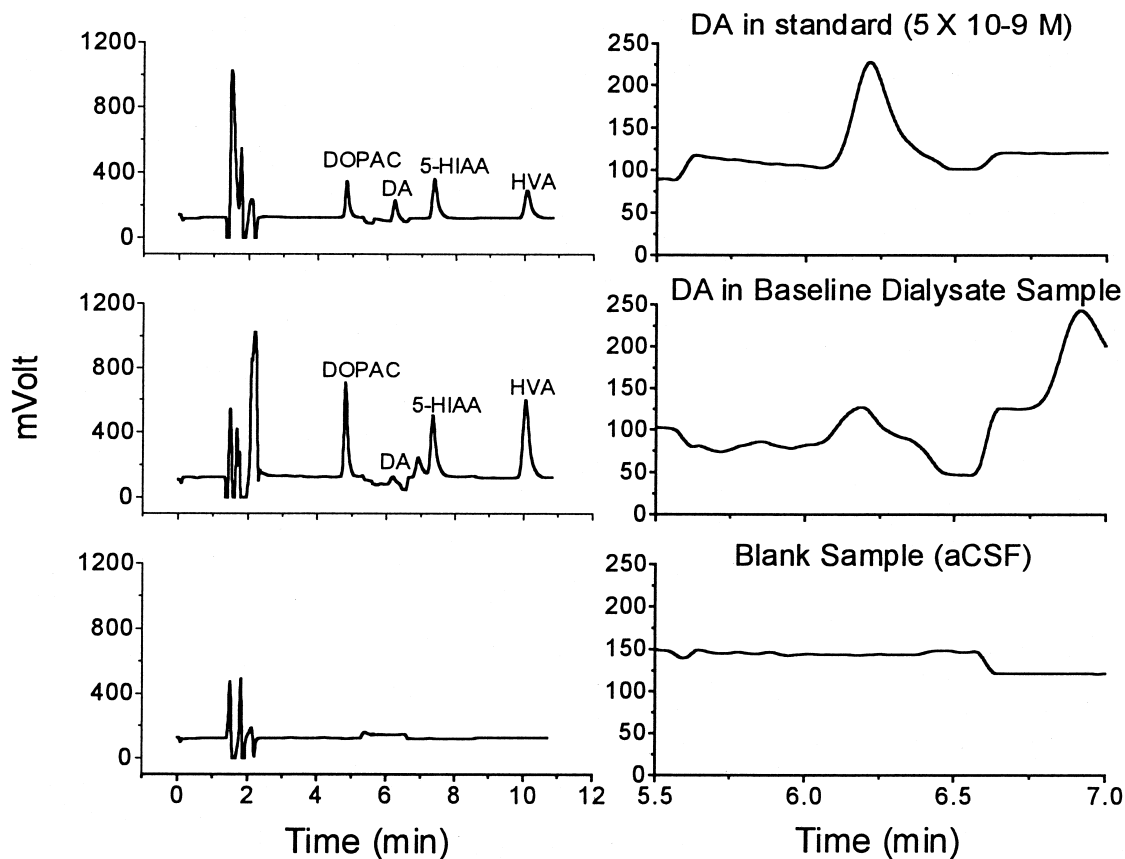


Fig. 1. Left: Representative chromatograms for the standard solution containing 5 nM DA (top), a baseline dialysate sample (middle) and a blank aCSF sample (bottom). Right: Magnification of the DA peaks in the corresponding chromatograms.

Post-hoc analysis demonstrated that 18-MC significantly increased both the maximum locomotor effect and the duration of locomotor activation in response to both 5 and

10 mg/kg morphine, without significantly altering the timecourses of locomotion in response to the other morphine doses tested (Fig. 3).

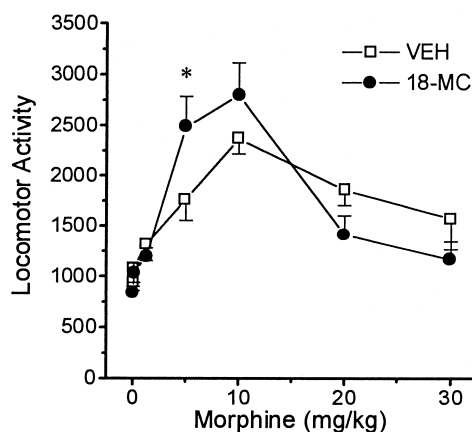


Fig. 2. Effects of pretreatment with 18-MC (40 mg/kg, i.p.; 19 h earlier) (solid symbols) or VEH (open symbols) on the dose-response relationship for locomotion in rats acutely treated with morphine (0–30 mg/kg). 18-MC shifted the dose-response curve for morphine-induced locomotion to the left of VEH-pretreated controls, indicating an increase in morphine potency by 18-MC. Each data point represents the mean locomotor response of 8–14 animals ($\pm$ S.E.M.). * $P < 0.05$, versus VEH, Duncan multiple-range post-hoc tests.

3.2. Repeated morphine locomotor study

Of the 94 rats tested, 54 displayed sensitization in response to repeated injections of 20 mg/kg morphine as defined by a decrease in the latency to locomotor activation [Group $\times$ Injection Number interaction: $F(4,368) = 5.89$, $P < 0.0005$; Group $\times$ Injection Number $\times$ Time interaction: $F(68,6256) = 2.46$, $P < 0.00001$; post-hoc tests]. As can be observed in Fig. 4 (open symbols), a difference between the activity of the two groups was apparent on Injection 1; compared to non-sensitized rats, sensitized rats displayed more robust locomotor depression in response to morphine during the first 90 min, whereas the locomotor excitatory responses of sensitized and non-sensitized rats did not differ from each other during the last 90 min of the session [Group $\times$ Time interaction: $F(17,2397) = 3.76$, $P < 0.0001$; post-hoc tests]. In contrast, on Injection 5, sensitized rats displayed significantly higher levels of locomotor excitation during the first 120 min following morphine injection, compared to non-sensitized rats [Group $\times$ Time interaction: $F(17,2391) = 4.27$, $P < 0.00001$;

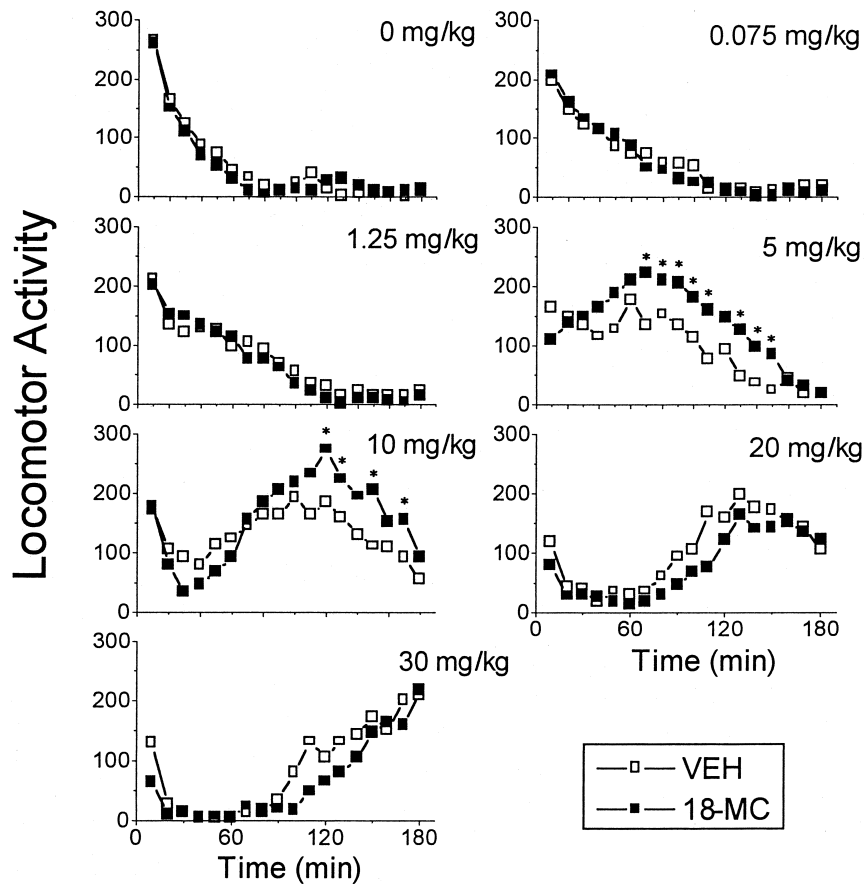


Fig. 3. Effects of pretreatment with 18-MC (40 mg/kg, i.p.; 19 h earlier) (solid symbols) or VEH (open symbols) on the timecourse of locomotor responding in rats acutely treated with morphine (0–30 mg/kg). 18-MC enhanced the maximum response and the duration of locomotion in response to both 5 and 10 mg/kg morphine. Each data point represents the mean locomotor response of 8–14 animals ($\pm$ S.E.M.). * P <0.05, versus VEH, Duncan's multiple-range post-hoc tests.

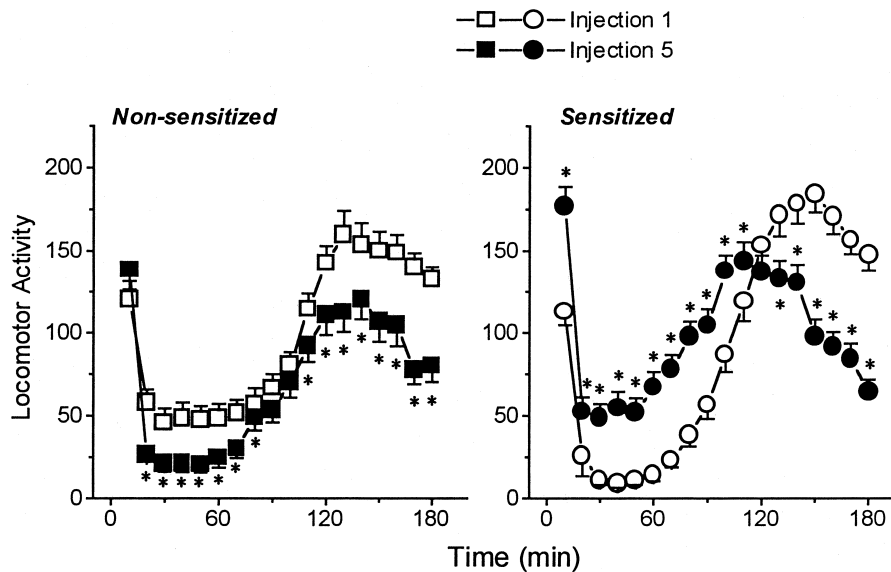


Fig. 4. Comparison of the time-courses of locomotor responding to 20 mg/kg morphine in sensitized ($N=54$; right) and non-sensitized ($N=40$; left) rats between Injection 1 (open symbols) and Injection 5 (closed symbols) of chronic morphine treatment (five, once daily, injections of 20 mg/kg). The onset of locomotor activation advanced in time from Injection 1 to Injection 5 of chronic morphine treatment in sensitized rats, but not in non-sensitized rats; this later group displayed a tolerance to morphine's locomotor-activating effects on Injection 5. Each point represents the mean ($\pm$ S.E.M.) of the groups at the indicated times after morphine injection. * P <0.05, versus Injection 1, Duncan's multiple-range post-hoc tests.

post-hoc tests]. As can be readily observed in Fig. 3, the latency to onset of locomotor activation advanced in time from Injection 1 to Injection 5 of repeated morphine treatment in the sensitized group [Injection Number $\times$ Time interaction, $F(17,2487)=5.42$, $P<0.0001$; post-hoc tests] whereas non-sensitized rats displayed an overall decline in locomotor activity across time between Injection 1 and Injection 5 of repeated morphine treatment [Injection Number $\times$ Time interaction, $F(17,1360)=34.29$; post-hoc tests]. These results indicate that the initial locomotor depression in response to an acute injection of morphine may serve as a predictor of the extent to which animals sensitize with repeated morphine administration.

On test day, morphine induced dose-dependent responding across time in both sensitized and non-sensitized rats [Dose effect: $F(3,78)=16.13$, $P<0.0001$]. As can be observed in Fig. 5 (left), in VEH-pretreated controls (squares), the dose-response curve for sensitized rats was shifted to the left of non-sensitized rats [Group $\times$ Dose interaction: $F(3,39)=3.27$, $P<0.04$]. In terms of total locomotor activity, sensitized VEH rats displayed maximum locomotor activation to 5 mg/kg morphine [Dose effect: $F(3,23)=4.56$, $P<0.02$, post-hoc tests], whereas maximum locomotor activation was observed at 10 mg/kg morphine in non-sensitized VEH rats [Dose effect: $F(3,16)=12.23$, $P<0.0005$, post-hoc tests]. These findings demonstrate that the sensitization expressed by sensitized VEH rats by the end of repeated morphine treatment persisted over the 3 days of morphine withdrawal and was present in the control rats on the test day.

18-MC (40 mg/kg, 19 h earlier) did not alter sig-

nificantly the dose-response curves in either sensitized or non-sensitized rats treated repeatedly with morphine [Group $\times$ Dose $\times$ Pretreatment interaction: $F(3,78)=1.75$, $P=0.16$]. However, inspection of the curves for the 18-MC pretreated groups (Fig. 5, right) suggested that, in contrast to VEH pretreated animals, no difference was apparent between the sensitized and non-sensitized groups pretreated with 18-MC. Statistical analysis supported this observation [Group $\times$ Dose interaction, $P=0.95$; Group $\times$ Dose $\times$ Time interaction, $P=0.99$]. Thus, whereas sensitization was still apparent in sensitized VEH rats on test day, sensitization was no longer expressed by sensitized 18-MC rats on test day. Thus it appeared that 18-MC pretreatment blocked the expression of sensitization in rats sensitized by previous morphine exposure.

3.3. Repeated morphine microdialysis study

The basal concentrations of dopamine and its metabolites, DOPAC and HVA, prior to morphine administration, did not differ as a consequence of either repeated morphine treatment (five, once daily injections of 20 mg/kg, i.p.) or 18-MC pretreatment (40 mg/kg, i.p., 19 h earlier) (Table 1).

Both repeated morphine treatment and/or 18-MC pretreatment significantly altered the responses of dopamine and its metabolites to a challenge injection of morphine [for dopamine, Group $\times$ Time interaction: $F(28,294)=3.40$, $P<0.0001$; for DOPAC, Group $\times$ Time interaction: $F(28,294)=1.61$, $P<0.03$; for HVA, Group $\times$ Time interaction: $F(28,294)=1.79$, $P<0.02$]. As can be observed in

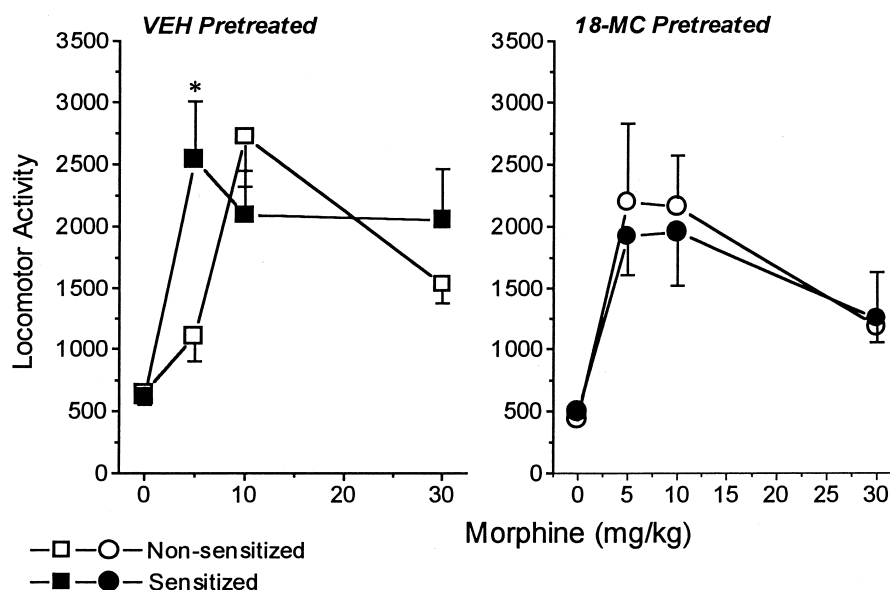


Fig. 5. Comparison of the dose-response curves for morphine-induced locomotion (0, 5, 10 and 30 mg/kg, i.p.) of sensitized (solid symbols) versus non-sensitized (closed symbols) chronic morphine treated (five, once daily injections of 20 mg/kg, i.p.) rats pretreated with either VEH (left) or 18-MC (40 mg/kg, i.p., 19 h earlier; right). Whereas a shift to the left in the dose-response function for locomotion was observed in VEH pretreated sensitized animals (indicating sensitization), no such shift was apparent in 18-MC pretreated sensitized animals (indicating a blockade of sensitization). Each data point represents the mean locomotor response of 5–7 animals ($\pm$ S.E.M.). * $P<0.05$ vs. non-sensitized group, Duncan's multiple-range post-hoc tests.

Table 1

Basal levels (pmol/10 μ l; mean $\pm$ S.E.M.) observed on Injection 1 of chronic morphine (MOR) treatment and on test day, 17–19 h after pretreatment with either 18-MC (40 mg/kg, i.p.) or vehicle

	Injection 1		Test day	
	Vehicle (n=6)	18-MC (n=6)	Vehicle (n=6)	18-MC (n=6)
Dopamine	0.015 $\pm$ 0.000	0.01 $\pm$ 0.002	0.013 $\pm$ 0.000	0.013 $\pm$ 0.000
DOPAC	5.160 $\pm$ 1.244	8.637 $\pm$ 2.493	6.189 $\pm$ 2.608	4.182 $\pm$ 1.161
HVA	2.445 $\pm$ 0.680	2.962 $\pm$ 0.893	2.081 $\pm$ 0.327	1.864 $\pm$ 0.921

Fig. 6, on Injection 1 of repeated morphine treatment, morphine administration (20 mg/kg, i.p.) produced a small increase in extracellular levels of dopamine in the nucleus accumbens that was significantly higher than baseline during the last sampling session (160–180 min post-injection) [Time effect: $F(14,154)=2.22$, $P<0.01$; post-hoc tests]. In addition, compared to baseline, increases in both DOPAC [Time effect: $F(14,154)=10.77$, $P<0.0001$] and HVA [Time effect: $F(14,154)=13.31$, $P<0.0001$] were observed (post-hoc tests) (Fig. 6, open squares in right panel). On test day, compared to their responses on Injection 1, VEH pretreated rats (Fig. 6, closed squares) displayed a sensitization of morphine-induced increases in dopamine levels in the nucleus accumbens which was apparent during the last 1.5 h of testing [Session $\times$ Time interaction: $F(14,140)=2.10$, $P<0.02$; post-hoc tests]. In addition, compared to Injection 1, a sensitization of both DOPAC and HVA levels were observed during the last 2 h of testing on test day [for DOPAC, Session $\times$ Time inter-

action: $F(14,140)=2.81$, $P<0.001$; for HVA, Session $\times$ Time interaction: $F(14,140)=3.15$, $P<0.0003$; post-hoc tests].

On test day, 18-MC pretreatment (40 mg/kg, 19 h earlier) reduced the dopamine response to morphine (20 mg/kg) in the nucleus accumbens, in comparison to both baseline during the test session and Injection 1 of repeated morphine treatment [Group $\times$ Time interaction: $F(28,294)=3.40$, $P<0.0001$; post-hoc tests] (Fig. 6). Thus, 18-MC prevents the dopamine-enhancing effects of morphine in the nucleus accumbens in rats repeatedly treated with morphine. Although 18-MC pretreatment did not abolish the DOPAC response to morphine, the levels of DOPAC did not differ from those of Injection 1 and 18-MC pretreated rats displayed lower levels of DOPAC in response to morphine during the final 40 min of testing, compared to VEH pretreated rats [Group $\times$ Time interaction: $F(28,294)=1.611$, $P<0.03$; post-hoc tests]. Thus, 18-MC blocked the sensitization of DOPAC levels pro-

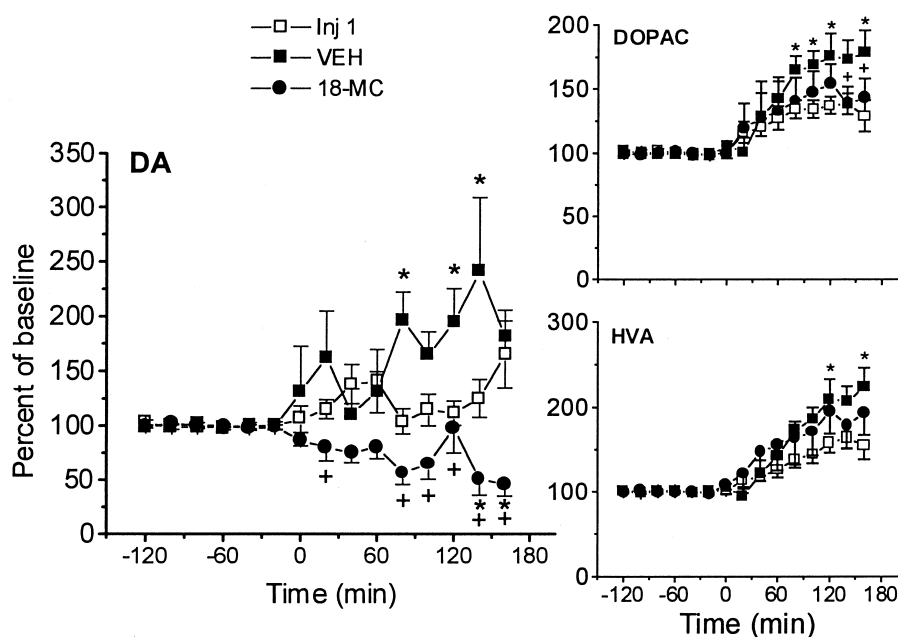


Fig. 6. Left: Comparison of the timecourse of extracellular dopamine in the nucleus accumbens of rats on Injection 1 of repeated morphine treatment (five, once daily injections of 20 mg/kg, i.p.; open squares) versus the test injection (20 mg/kg, i.p.; $n=12$), prior to which rats were pretreated (19 h earlier) with either 18-MC (40 mg/kg, i.p.; $n=6$; closed circles) or VEH ($n=6$; closed squares). Right: Comparison of the extracellular levels of DOPAC (top) and HVA (bottom) during the same microdialysis sessions. 18-MC completely blocked the increase in dopamine in the nucleus accumbens produced by morphine on test day and blocked the expression of sensitized levels of DOPAC. 18-MC did not significantly alter accumbal HVA levels. *, $P<0.05$ vs. Injection 1; +, $P<0.05$ vs. VEH. Duncan's multiple range post-hoc tests.

duced by repeated morphine administration. In contrast, 18-MC pretreated rats displayed higher levels of HVA in response to morphine during the last 1.5 h of testing, compared to Injection 1, but their HVA responses did not differ at any time from VEH pretreated rats [Group $\times$ Time interaction: $F(28,294)=1.79$, $P<0.02$; post-hoc tests]. Thus, 18-MC appears to exert its effects on the sensitized dopamine and DOPAC responses in the nucleus accumbens to morphine of morphine-experienced rats, without altering the sensitized HVA response in this region.

4. Discussion

The present results show that pretreatment (40 mg/kg, 19 h earlier) with the potential anti-addictive agent, 18-MC, differentially alters the dose–response relationship for morphine-induced locomotion depending on the previous morphine history of the animal; 18-MC pretreatment increased the potency of morphine to elicit locomotor activation in acute morphine treated rats but blocked the expression of locomotor sensitization in rats previously sensitized by repeated morphine experience. Consistent with this latter finding, 18-MC (40 mg/kg, 19 h earlier) completely abolished the sensitized dopamine response to morphine and blocked the expression of sensitization of DOPAC levels in the nucleus accumbens in chronic morphine-treated rats. These findings indicate that 18-MC differentially alters the pharmacokinetic and/or pharmacodynamic properties of morphine, depending on the previous morphine history of the animal, and that these effects, at least in morphine-experienced rats, may reflect an attenuation of accumbal dopamine transmission.

Previous studies using the prototypical iboga agent, ibogaine, demonstrated that ibogaine reduces the efficacy of morphine to elicit locomotion in acute morphine-treated rats [35,42,43]. Thus, it appears that, with respect to morphine's acute locomotor activating effects, 18-MC interacts with morphine in a manner different from its parent compound. This finding is not surprising given that differences with respect to the effects of ibogaine and 18-MC on drug responses have been noted in several studies (for reviews of this issue see [18,19]). Presumably, these differences are related to differences in the receptor binding profiles for ibogaine and 18-MC [17,18] and especially for morphine-induced behaviour, differences in affinities for the δ opioid receptor ($\mu\text{M } K_{i, 18\text{-MC}} = 3.5 \pm 0.05$ versus $\mu\text{M } K_{i, \text{IBO}} > 100$) [9,33,37]. Although the present study cannot distinguish which, if any, receptor-binding differences are responsible for the differential effects of ibogaine and 18-MC on acute morphine-induced locomotion, given the complexities of their receptor binding profiles, different combinations of receptor-mediated mechanisms are likely to be involved. This suggestion is exemplified by recent observations that, despite differences in their effects on acute morphine-induced locomotion,

ibogaine [35,36,43] and 18-MC [21] produce qualitatively similar attenuations of dopamine levels in the nucleus accumbens and the striatum in response to an acute injection of morphine. Thus, it appears that the effects of 18-MC on acute morphine-induced locomotion are separable from this drug's effects on acute morphine-induced dopamine release/synthesis, a finding consistent with recent preliminary studies of the interactions between 18-MC and cocaine [18,19].

The effects of ibogaine on the dose–response relationship for morphine-induced locomotion has yet to be determined in morphine-sensitized animals; nonetheless previous ibogaine studies demonstrated that ibogaine produces a greater decrease in morphine-induced locomotion in rats with prior morphine experience (2–4 injections of 30 mg/kg, i.p.) [43]. The present findings are consistent with previous ibogaine results; 18-MC blocked the expression of locomotor sensitization in rats sensitized by prior morphine experience. Thus, it appears that the effects of 18-MC pretreatment on morphine-induced locomotion depend on the previous drug history of the animal, as per previous indications for ibogaine [6,43,57]. This raises the possibility that 18-MC and ibogaine, while possibly acting through different mechanisms to influence acute morphine-induced locomotion, may be acting via a common mechanism(s) to reduce locomotion in rats with previous morphine experience.

It is well-established that the repeated systemic administration [for review, [29]] and/or intravenous self-administration [11] of morphine, and other addictive substances, can induce the expression of sensitization of locomotor behaviour. This behavioural effect is putatively mediated by the development of enhanced dopamine transmission in the nucleus accumbens (for review see [29]), caused by repeated actions of morphine at opioid receptors located on GABAergic neurons in the ventral tegmental area, the activation of which leads to a disinhibition of the activity of mesolimbic dopamine neurons [26,27,30]. Opioid receptors located on GABAergic or enkephalin-containing neurons in the nucleus accumbens [8] do not appear to be involved in the development of either morphine-induced dopamine or locomotor sensitization (for review see [29]). In the present study, 18-MC completely abolished the dopamine response and blocked the sensitized DOPAC response to morphine of rats with previous morphine experience. That no effects of 18-MC were observed on morphine-induced increases in extracellular levels of HVA in the accumbens suggests that 18-MC pretreatment produced a protracted effect on dopamine release mechanisms, but did not alter the changes in dopamine synthesis produced by repeated morphine administration.

Although not identical, the neurochemical findings of the present study are consistent with previous reports for ibogaine in morphine-experienced rats [44]. In combination, these findings suggest that one common mechanism through which 18-MC and ibogaine exert some of their

effects on morphine-induced locomotion in morphine-experienced animals is related to a blockade or reversal of the neuroadaptations mediating enhanced dopamine release from dopamine terminals in the nucleus accumbens. Indeed, repeated, intermittent, morphine administration is known to cause a variety of alterations in both the number of receptors for which both 18-MC and ibogaine show moderate binding affinity (e.g., [18,19]) and in the signal transduction pathways associated with these receptors. For instance, changes in μ , and κ receptor expression/function either directly by morphine [9,22,37,48,55], or indirectly by effects of repeated morphine on endogenous opioid transmission e.g., [7,48] may prove to be the most relevant to the similar effects of both ibogaine and 18-MC on the behavioural and neurochemical consequences of repeated morphine administration; these receptors have been implicated in both the dopamine-releasing properties e.g., [10,24,26,32,54] and locomotor-sensitizing effects e.g., [23,27,33,53] of morphine.

A recent study conducted in our laboratory demonstrated that 18-MC shifts the dose–response curve for morphine self-administration downward [34], indicating that 18-MC attenuates the reinforcing efficacy of morphine. Several laboratories have demonstrated that chronic drug self-administration can induce the expression of behavioural and/or neurochemical sensitization e.g., [11,12,45] and several theories have implicated the phenomenon of sensitization in the mediation of drug reward/reinforcement, drug-seeking behaviour and relapse in addiction e.g., [3,28,51]. Given (1) the putative role for mesolimbic dopamine transmission in the mediation of the appetitive, rewarding/reinforcing or incentive motivational effects of opiate drugs [2,3,31,32,59], but see [12], and (2) that 18-MC blocked both the expression of the sensitized locomotor and dopamine responses in chronic morphine treated rats in the present study, raises the intriguing possibility that 18-MC might attenuate morphine self-administration by interfering with the expression of sensitization of dopamine transmission caused by repeated morphine administration, possibly via actions at opioid receptors in the ventral tegmental area e.g. [3,52,53].

In summary, the present study demonstrated that the effects of pretreatment with the synthetic iboga alkaloid congener and potential anti-addictive drug, 18-MC (40 mg/kg, 19 h earlier), differentially alters the dose–response function for morphine-induced locomotion in acute versus repeated morphine treated rats; 18-MC increased the potency of morphine to elicit locomotion in rats treated acutely with this opioid but blocked the expression of morphine-induced sensitization in rats sensitized by prior morphine treatment. Based on previous microdialysis studies, it does not appear that the effects of 18-MC on acute morphine-induced locomotion are related to its effects on morphine-induced increases in mesolimbic dopamine transmission; however, the present results indicate that the ability of 18-MC to block morphine-induced

locomotor sensitization may be mediated, at least in part, via a blockade of dopamine release in the nucleus accumbens in morphine-sensitized animals. Given the putative role for sensitization of dopamine transmission in the nucleus accumbens in the mediation of various aspects of drug addiction, it is postulated that the potential anti-addictive efficacy of 18-MC might be related to an ability to restore normal functioning to a morphine-hypersensitive mesolimbic dopamine system, thereby reversing the pathological psychobiological consequences of repeated morphine administration.

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